



Patent  
Attorney's Docket No. 010091-001

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Patent Application of	)	
	)	
Richard SCHLEGEL et al.	)	Group Art Unit: 1813
	)	
Application No.: 08/216,506	)	Examiner: A. Caputa
	)	
Filed: March 22, 1994	)	
	)	
For: PAPILLOMAVIRUS	)	
VACCINE	)	
	)	

**DECLARATION PURSUANT TO 37 C.F.R. § 1.132**

Honorable Commissioner of Patents and Trademarks  
Washington, D.C. 20231

Sir:

(1) I, C. Richard Schlegel, M.D., Ph.D., declare and state that I am a citizen of the United States residing at 3 Elmwood Court, Rockville, Maryland 20850.

(2) I was awarded an M.D./Ph.D. from Northwestern University Medical School, Chicago, Illinois in 1975. Subsequently I completed residency training in Pathology at the Brigham Hospital, Harvard University, and was board-certified in Anatomic Pathology by the American Board of Pathology in 1979. My studies on the uptake and neutralization of viruses by antibodies began in 1980 when I was doing a post-doctoral fellowship in the Laboratory of Molecular Biology at the National Institutes of Health (Laboratory Chief: Dr. Ira Pastan).

(3) I was an Associate Professor in the Department of Pathology at Georgetown University Medical School from 1990-1992 and have since been promoted

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to Professor (1992-present). In addition, I am also a Professor in the Department of Obstetrics and Gynecology at Georgetown University Medical School. I have been the director of the Molecular Pathobiology Program in the Department of Pathology at Georgetown University Medical School from 1991 to the present date. Further, I have been the Director of the Ob/Gyn Oncology Research Program in the Lombardi Cancer Center, Georgetown University School of Medicine from 1991 to the present date.

(4) I am a co-inventor of the above-identified application. I have reviewed the prosecution history in the parent application, U.S. Serial No. 07/903,109, filed on June 25, 1992. Based on my review of the Office Actions issued in connection with this application, it is my understanding that the Examiner is of the opinion that the disclosure contains insufficient information for one skilled in the art to conclude that recombinant produced conformationally correct papillomavirus L1 proteins may be used as an effective immunogen for conferring immunity against papillomavirus infection in susceptible mammals. Specifically, the Examiner is of the opinion that the application does not contain sufficient proof that papillomavirus conformationally correct L1 proteins may be used as a vaccine against any papillomavirus and in particular for conferring protection against human papillomaviruses given the absence of *in vivo* data. I disagree with the Examiner's contentions because the xenograft and C127 neutralization *in vitro* assays contained in the subject application are conventionally used by those skilled in the art to establish the *in vivo* efficacy of putative papillomavirus immunogens. I further disagree with the Examiner's contentions

because the application contains convincing evidence that L1 proteins expressed in eukaryotic host cells may be expressed in conformationally correct form which mimic the antigenicity of the intact viral particle. This was established by the experiments contained in the application which demonstrate that the recombinant HPV-1 L1 protein expressed in COS cells specifically binds to monoclonal antibodies which are specific to conformational epitopes of the HPV-1 L1 protein. Most importantly, I disagree with the Examiner's conclusion that there is insufficient proof in the application as-filed that papillomavirus conformationally correct L1 proteins may be used as a vaccine against papillomavirus. However, to further refute the Examiner's conclusion, I am submitting new experimental data herein which provides further proof in support of the claimed invention. In these additional experiments, it is shown conclusively that conformationally correct L1 proteins of the canine oral papillomavirus (COPV) can protect dogs (with 100% efficacy) against infection by high-titer COPV. Moreover, based on the extensive similarity between COPV and HPV's, this experimental data further provides convincing evidence that conformationally correct HPV L1 proteins may be used to protect humans against the homologous human papillomavirus.

(5) The following three experiments were conducted by me or under my direction, and are believed to provide conclusive *in vivo* evidence that conformationally correct L1 papillomavirus proteins may be used to confer immunity against papillomavirus infection in susceptible animals. The first experiment demonstrates that the administration of a formalin inactivated canine oral papilloma homogenate

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containing the COPV L1 capsid protein confers immunity in Beagle dogs against mucosotropic canine oral papillomavirus (COPV), which naturally infects 25% of weanling Beagles in non-vaccinated animals. This vaccine has been used routinely for several years in approximately 60,000 dogs with complete protection and with no adverse effects. The second experiment shows that administration of serum immunoglobulin fractions obtained from Beagles which had been immunized with crude wart extract are capable of passively protecting non-vaccinated Beagles. The third experiment is the most important and conclusively shows that recombinant conformationally correct COPV L1 proteins expressed in baculovirus infected Sf9 cells can be used as a successful vaccine for protecting against COPV infection. The results of these three experiments together demonstrate convincingly that conformationally correct L1 proteins may be used as an effective vaccine for conferring protection against COPV infection.

COPV and the HPV's associated with human malignancy are highly similar: they exhibit similar genetic organization, viral structure, capsid protein sequences, and selectively infect a mucosal site of infection. Therefore, as stated in the Cossman and Pearson § 1.132 Declarations, both of which persons are experts in the field, as well as in the summary report of a panel of NIH scientists who reviewed the NIH Schlegel research grant, which is attached to this declaration, it is reasonable to assume that the success of the COPV vaccine (demonstrated in the new submitted data *infra*) would be

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predictive of the efficacy of a human papillomavirus vaccine containing conformationally correct HPV L1 proteins.

## **EXPERIMENT ONE**

### **Abstract**

This experiment describes the successful use of a formalin-inactivated canine oral wart homogenate as a vaccine to prevent infection by COPV in Beagle dogs. In this experiment, 26 dogs received doses of phosphate buffered saline (PBS) intradermally, and 99 dogs received two doses of a formalin-inactivated vaccine containing approximately 50 ng of COPV L1 capsid protein. One month after the second dose, all 125 dogs were challenged with infectious COPV by scarification of the oral mucosa. All of the control group developed papillomavirus by 6-8 weeks after challenge. By contrast, none of the dogs immunized with the formalin-inactivated vaccine containing COPV L1 conformational capsid protein developed perceptible papillomas. The methodology of this experiment is described in detail below.

### **Materials and Methods**

Equal numbers of male and female outbred Beagle dogs were obtained and maintained at Marshall Farms in North Rose, New York. The animals were vaccinated for parvovirus (31, 38, 45, and 59 days of age Northwest Tennessee Veterinary

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Services, Dresden, Tennessee); canine parainfluenza-*Bordatella bronchisepticum* (26 days of age, Intra-Trac-II, Schering-Plough Animal Health, Omaha, N.A.); mink distemper (69 days of age, Distem-R TC, Schering-Plough Animal Health); canine distemper, adenovirus type-II, parainfluenza, parvovirus, and *Leptospira* (105 days of age, Duramune, DA<sub>2</sub> LP + PV, Fort Dodge Laboratories, Fort Dodge, IA); and rabies, (100 days of age, Imrab-1, Pitman-Moore, Mundelein, IL.). Blood was drawn routinely at 120 days of age for CBC, reticulocyte count, and prothrombin times.

The dogs were nursed by their dams until 8 weeks and then put on a commercial diet. After weaning, the dogs were housed in open sheds in wire cages suspended above the ground. The dogs had access to tap water *ad libitum*. The dogs were on a natural light cycle.

#### Vaccine Preparation

Twenty-five (25) dogs were routinely inoculated as described below with live COPV in order to induce papillomas that produced infectious virions. After inoculation, the papillomas were removed surgically eight weeks after induction by scarification. The resultant papillomas were frozen in plastic vials and stored at -70°C until used. For vaccine production or preparation of the challenge inoculum, papillomas were thawed, placed on two aluminum plates, and macerated by hammering the plates together. The material was then placed in a blender with chlorinated tap water (2 grams of tissue into 100 ml total of water, 2% w/v) and homogenized for 10 minutes

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at room temperature. The homogenate was then passed twice through cheese cloth to remove large particulates and then frozen at -70°C. This preparation was thawed slowly to room temperature and then used to challenge dogs to induce productive oral papillomas or inactivated by the addition of 8 ml of neutral buffered 10% formalin to 240 mls (1:30 v/v) of the filtered homogenate, stored at 4°C for 48 hours, and then used as the vaccine. This crude vaccine contained COPV L1 protein at concentrations ranging from 50-1000 ng/ml as determined by quantitative immunoblotting and ELISA techniques.

#### **Animal Vaccination**

All dogs were injected intradermally twice, at 8 and 10 weeks of age. For each injection, 0.2 ml of vaccine formulation was injected into the foot pad of the dew claw (phalanx) using a TB syringe with a 26 gauge needle. Twenty-six Beagle dogs received phosphate buffered saline, pH 7.4, as a placebo. Ninety-nine Beagle dogs received the formalin-inactivated vaccine in the same manner.

#### **Challenge of Vaccinated Dogs and Control Groups**

All 125 dogs were then challenged with infectious live COPV by scarification with a wire brush on the dorsal buccal and maxillary mucosa. Challenge with infectious virus was performed one month after the second dose of vaccine or placebo solution.

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After challenge, each dog was examined daily by a clinical veterinarian or a trained veterinary technician for eight weeks.

### **Results**

All of the control group dogs (26/26) which were injected with PBS and challenged with infectious COPV developed oral papillomas between six and eight weeks following exposure to the virus. By contrast, none of the dogs which were injected with the formalin-inactivated preparation (0/99) developed clinically evident oral papillomas. These results are believed to provide persuasive *in vivo* evidence that vaccination with wart extract containing conformationally correct L1 proteins may be used to protect Beagles against COPV infection. Additionally, given the substantial genetic and structural similarities between COPV and HPV, these results suggest that similar approaches may be applied for the prevention of HPV infections.

### **EXPERIMENT TWO**

#### **Abstract**

In this experiment, serum obtained from the above vaccinated weanling dogs was passively transferred to naive dogs. The recipient non-vaccinated dogs (which had



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received the immune serum transfer) were then evaluated for protection against COPV infection.

### **Materials and Methods**

Serum samples were harvested by phlebotomy from either non-immune 10 week old naive beagle weanlings or from immune 12 week old weanlings which had been vaccinated with a crude COPV wart vaccine on weeks 8 and 10 following birth as described in Experiment One. The serum immunoglobulin fraction was obtained from both groups by ammonium sulfate differential precipitation and dialysis against phosphate buffered saline.

After the immunoglobulin solutions were obtained from the non-immune and the immune dogs, these solutions were then administered intravenously to two groups of four dogs intravenously over a 20 minute period at a dosage of 200 mg/kg. Additionally, a control group of 4 dogs was administered lactate Ringers solution intravenously over a 20 minute period at a dosage of 200 mg/kg. These three groups of dogs were then challenged with infectious live COPV by scarification as described in Experiment One.

### **Results**

The results of this experiment are illustrated in Figure 1. As summarized therein, all the dogs which were administered lactate Ringers solution as well as the dogs which

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were administered non-immune dog serum developed papillomas after challenge with live infectious COPV. By contrast, none of the dogs who received the immune dog serum from the dogs which had been vaccinated with the crude COPV wart extract showed any signs of papillomas after challenge with live infectious COPV.

Therefore, these results provide evidence that immunity induced by the wart vaccine is mediated by immunoglobulins and that complete protection of animals can be achieved by these antibodies without the need or cooperation of cellular immune responses. This is a critical element in the design of a papillomavirus vaccine, whether it be for COPV or HPV.

Also important is the observation that the dose of challenge COPV used in this study was extremely high. Usually dogs require 6-8 weeks in order for tumors to become evident. However, in this specific experiment, a concentrated preparation of wart extract was used which generated tumors within 3 weeks. Thus, even when animals are challenged with extraordinarily high titers of virus, they are protected by passively transferred antibodies.

### **EXPERIMENT THREE**

#### **Abstract**

The previous two experiments described two critical elements in the development of a COPV vaccine: (1) the success of a formalin-inactivated wart extract containing COPV L1 proteins for conferring immunity against COPV in Beagle dogs and (2) the use of serum obtained from the above vaccinated dogs for the passive transfer of immunity. The final critical element, which is the object of experiment three, is to show that the isolated, conformationally-correct form of COPV L1 protein is the essential element in the vaccine which elicits immunity and that the COPV L1 protein is sufficient in itself in inducing protective immunity.

#### **Materials and Methods**

The following experiment relates to the use of conformationally correct recombinant COPV L1 proteins, in particular COPV L1 proteins expressed in recombinant baculovirus virus infected Sf9 cells, as a vaccine against COPV in Beagle dogs.

In this experiment, 40 dogs were vaccinated at 8 and 10 weeks of age with 0.2 ml of several vaccine formulations. The injections were performed in the foot pad as described previously in Experiment 1. The recombinantly-expressed L1 protein was examined in the electron microscope and found to assemble into virus-like particles

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and, more importantly, to react with antiserum that was specific for COPV conformational capsid surface epitopes. The first control group of dogs was mock-vaccinated with phosphate buffered saline (Group I), and the second group of dogs was vaccinated with formalin-fixed wart homogenates (Group II) as described in Experiment One. The third group was vaccinated with 20  $\mu$ g L1 protein contained in phosphate buffered saline (Group III), the fourth group with 20  $\mu$ g of L1 protein in PBS containing alum (Group IV), and the fifth group with 20  $\mu$ g L1 protein in QS21 adjuvant (Group V).

Two weeks after completing the second administration of vaccine, all the animals were challenged with live, infectious COPV by scarification with a wire brush as in Experiment One. Dogs were then evaluated weekly after challenge to detect oral papillomas for 10 weeks.

### **Results**

In the control group of beagles (given phosphate buffered saline for vaccination), six of eight animals (Group I) developed oral tumors. By contrast, none (zero of thirty-two) of the dogs which were injected with formalin-fixed wart extract or any of the recombinant L1 protein- containing compositions showed any signs of oral tumors after challenge.

These results are summarized in Figure 2 and conclusively establish that recombinant conformationally correct L1 proteins may be used as an effective vaccine against COPV in Beagle dogs. This experiment also indicates that COPV L1 protein is

sufficient (in the absence of viral L2 capsid protein as well as other cellular proteins in the wart extract) to completely protect against infectious COPV challenge. Moreover, given the substantial similarities between COPV and human papillomavirus, these results further establish that conformationally correct human papillomavirus L1 proteins may be used as an effective vaccine against human papillomavirus infection.

To further establish the importance of L1 conformation, the antibody response against both linear and conformational COPV L1 epitopes and was compared after the first vaccination, after the second vaccination, and after challenge with infectious COPV. These results are summarized in Figure 3 and Figure 4. It can be clearly seen from these figures that the Beagle dogs which were inoculated with the wart extract or with the recombinant conformationally correct COPV L1 proteins exhibit a substantial antibody response against COPV conformational epitopes. By contrast, the control group exhibited virtually no change in the antibody response to conformational epitopes after challenge.

While vaccinated animals clearly developed an immune response to conformational L1 epitopes, they failed to develop a significant response to linear (non-conformational epitopes) as demonstrated in Figure 3. This provides further evidence that antibodies to linear epitopes are not involved in protection.

Group 4 animals, which were inoculated with the recombinant L1 protein in alum, had the highest linear epitope antibody response. This suggests that the alum

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adjuvant may partially affect the L1 protein's conformational structure, thereby exposing linear L1 epitopes to the dog's immune system.

(6) I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date \_\_\_\_\_

\_\_\_\_\_  
C. Richard Schlegel

## FIGURE 1

### PROTECTION OF DOGS AGAINST COPV INFECTION BY THE PASSIVE TRANSFER OF IMMUNOGLOBULIN

TREATMENT PROCEDURE	Number of dogs with tumors	Number of dogs
Infused with lactate Ringers solution	4	4
Infused with non-immune dog serum, 200 mg/kg	4	4
Infused with immune dog serum, 200 mg/kg	0	4

## FIGURE 2

### DOG VACCINATION STUDIES UTILIZING CONFORMATIONALLY-CORRECT L1 PROTEIN PURIFIED FROM RECOMBINANT-BACULOVIRUS INFECTED Sf9 CELLS

Vaccination Procedure	#dogs with oral tumors
Buffer	6/8
Formaline-fixed wart extract	0/8
L1	0/8
L1 + alum adjuvant	0/8
L1 + QS21 adjuvant	0/8



Exp. COPV#t:1 VLP  
 4/8/94: Coating Ag-Isolated COPV particles. Dog sera: 1:100 dilutions of pooled (n=8) samples.  
 KGP 2° (anti-IgG) at 1:200. Rabbit sera at 1:500, KGP 2° at 1:1000. 30 minute reading  
 Normal Rabbit serum was 0.080.

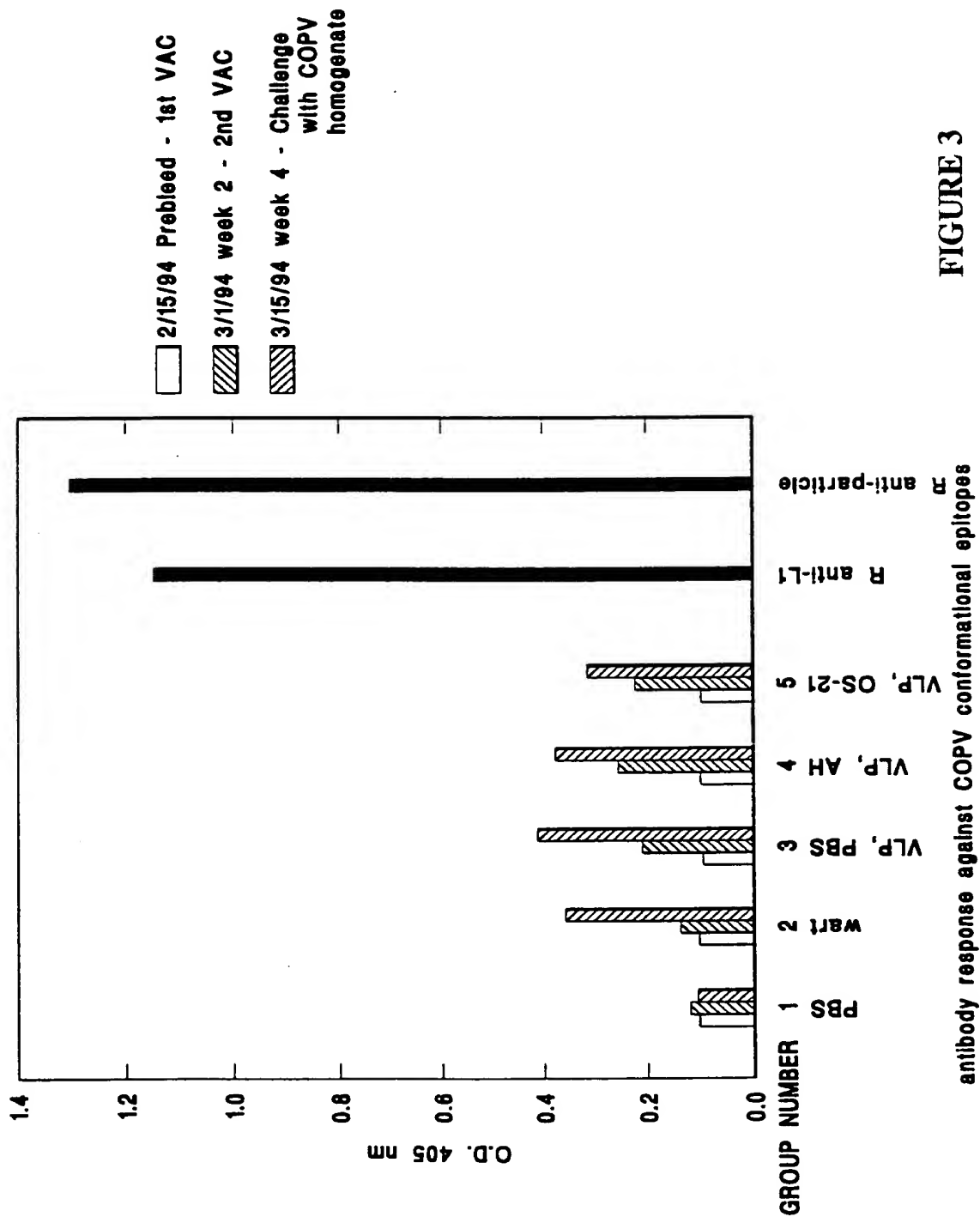


FIGURE 3

Exp. COPV#1:L1 VLP  
 4/6/94: Coating Ag=Recombinant L1(Insoluble). Dog sera: 1:100 dilutions of pooled (n=8) samples.  
 Bethyl 2° (anti-IgG) at 1:500. Rabbit sera at 1:500. KGP 2° at 1:1000. 30 minute reading  
 Normal Rabbit serum was 0.080.

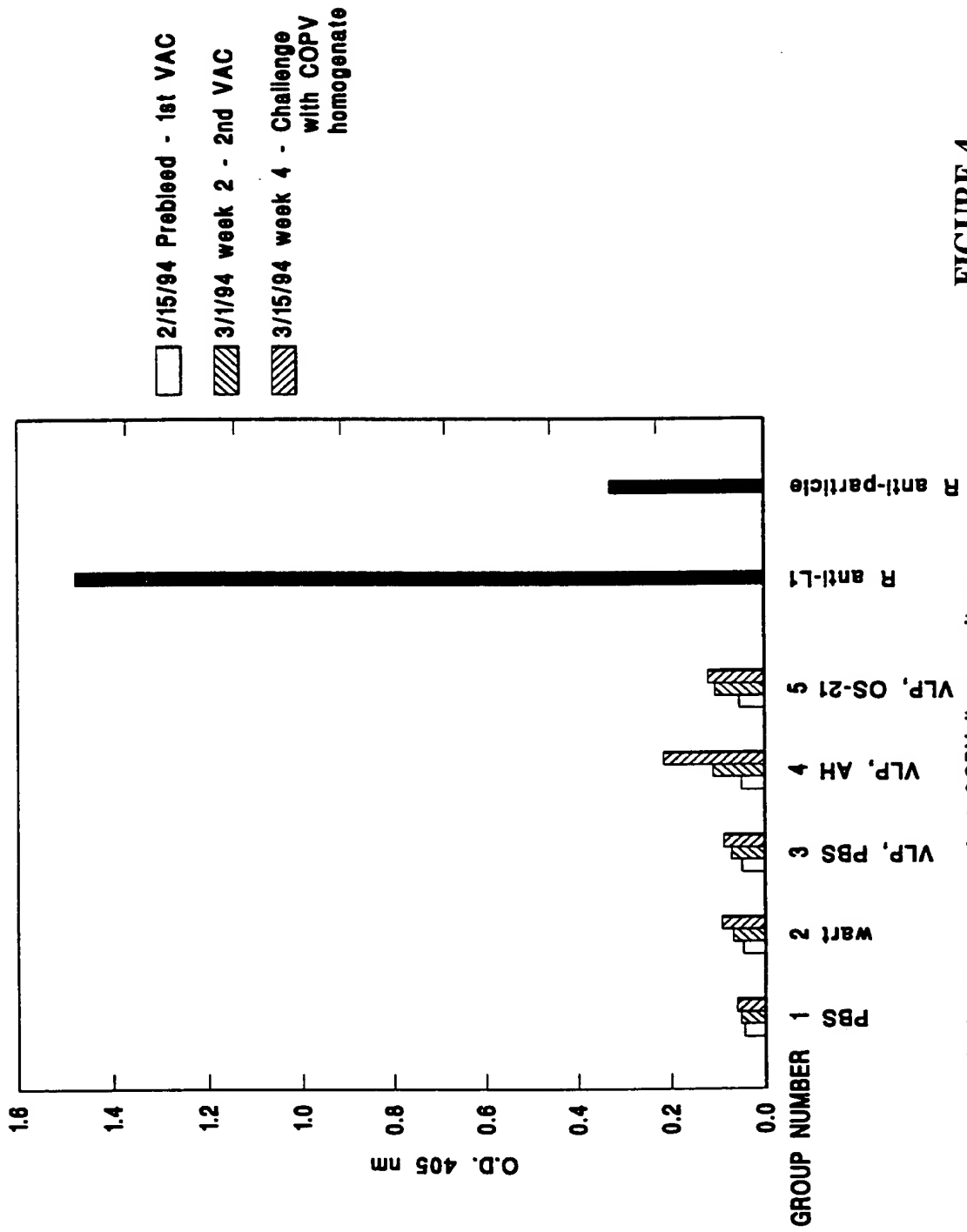


FIGURE 4

antibody response against COPV linear epitopes

DR. MAY WONG  
 BIO CARCINOGENESIS (DCE) (Privileged Communication)  
 301 496-1953

## SUMMARY STATEMENT

Application Number: 1 R01 CA57994-01

Review Group: SRC (66)  
 SPECIAL REVIEW COMMITTEE

Meeting Dates: IRG: FEB/MARCH 1992  
 COUNCIL: MAY 1992

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 CA91-28

Investigator: SCHLEGEL, RICHARD Degree: MD

Organization: GEORGETOWN UNIVERSITY  
 City, State: WASHINGTON, DIST OF COL

Requested Start Date: 07/01/92

Project Title: CANINE ORAL PAPILLOMAVIRUS: MODEL FOR A VACCINE

IRG Action: 1/  
 Human Subjects: 10-NO HUMAN SUBJECTS INVOLVED  
 Animal Subjects: 30-ANMLS INV.-VERIFIED, NO IRG CONCERNS OR COMMENT.  
 Gender: 60-NOT CLINICAL RESEARCH  
 Minority: 70-NOT CLINICAL RESEARCH

PROJECT YEAR	DIRECT COSTS REQUESTED	DIRECT COSTS RECOMMENDED	ESTIMATED TOTAL COST
01	116,848	115,048	186,025
02	148,482	142,048	229,682
03	154,421	142,048	229,682
04	<u>160,598</u>	<u>142,048</u>	<u>229,682</u>
TOTAL	580,349	541,192	875,071

Resume: The ultimate goal of the studies in this application is to produce a vaccine for canine oral papillomavirus (COPV), which can serve as a model for the development of a human papillomavirus (HPV) vaccine. This is a well written and carefully conceived proposal by an investigator who has made important contributions to the study of the cell and molecular biology of papillomavirus replication. A canine model will be explored to examine natural and experimental papillomavirus infection and to assess various vaccine regimens for their efficacy in inhibiting infection and promoting disease regression. This is one of the best, if not the best, system for such a study provided that sufficient amounts of the relevant viral immunogens can be obtained. Due to the similarities between COPV and HPV with respect to viral genetic organization, viral structure, capsid protein sequences and mucosal site of infection, there is a high probability that the findings generated in the beagle system will have direct applicability to the development of a human papillomavirus vaccine. Consequently, support for four years is recommended.

Date Released: 03/25/92

Date Printed: 03/25/92

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**DESCRIPTION**

Human papillomaviruses, which infect the genital tract mucosa, play a critical role in the development of cervical cancer. For example, the majority (95 percent) of human cervical carcinomas contain and express HPV DNA. Moreover, the expression of two viral oncoproteins, E6 and E7, appear to be critical for cellular transformation. Despite our detailed knowledge concerning the molecular mechanism of action of these oncoproteins, little information exists on the biology of papillomavirus infection. This includes the identity of viral receptors, control of viral replication and assembly, and the host immune response to virus and virus-transformed cells. The aforementioned deficiencies in knowledge derive from the lack of an in vitro system for propagating papillomavirus (which requires highly differentiated keratinocytes) as well as the lack of an available animal model to analyze the host factors which participate in the response to mucosal infection by papillomavirus.

The applicant and his co-investigators propose to study the biology of COPV infection in a beagle colony, which exhibits a high incidence of oral wart formation as a consequence of viral infection. The intent is to delineate the role of antibodies in the resistance of animals to infection that can be produced by injection of wart extracts. Such preparations are known to contain viral particles. The L1 and L2 genes of COPV will be sequenced and cloned into expression vectors. Preliminary data indicate that these vectors produce viral proteins which retain their native conformation. The viral capsid proteins will be used to screen immune animal sera for the presence of L1- and L2-specific antibodies as well as to induce immunity in susceptible animals. Optimal conditions will be developed for inducing immunity. The ability of L1 and L2 antibodies to inhibit COPV-induced tumors will be evaluated using purified virions derived from wart tissue or from virus-producing tumors produced in nude mice. Finally, monoclonal antibodies will be generated against intact virions in order to define the molecular location of conformational neutralizing epitopes on COPV.

The specific aims of this investigation are to: 1) examine sera from beagles immunized with wart extracts containing COPV using purified virions or expressed L1 and L2 proteins; 2) sequence L1 and L2 genes and clone them into pSVL and baculovirus expression vectors; 3) develop monoclonal antibodies against purified COPV virions and L1 and L2 proteins. The conformation, neutralizing ability and use of expressed proteins for affinity purification will be examined; 4) vaccinate beagles with virions or L1 or L2 proteins followed by challenge with COPV. The appearance of natural infections will be observed and optimal vaccination conditions will be determined; 5) examine temporal production of specific IgG, IgM and IgA antibodies; and 6) develop two in vitro systems for the analysis of viral neutralization, transformation of fibroblasts or keratinocytes and tumor formation of oral mucosa implanted in the nude mouse.

**CRITIQUE**

This is a carefully conceived and logical proposal by an investigator who has made important contributions to the study of the cell and molecular biology of papillomavirus replication. With this proposal, the applicant now moves more actively into the field of papillomavirus immunology and pathogenesis. The

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Richard Schlegel, M.D., Ph.D.

overall aim of the investigation is to expand studies in a canine model of natural papillomavirus infection in order to evaluate the immunogenic potential of viral capsid preparations.

The canine model of natural papillomavirus infection possesses several advantages. These include lesion development on a mucosal surface and the possibility of examining immunity in a naturally infected population. A possible disadvantage of the canine model is the lack of reagents to study parameters of the immune response in great detail. Despite this limitation, the principal investigator has focused on what can be done to exploit this model. The proposed studies to evaluate antibody responses against intact COPV virions and virion constituents are straightforward. The applicant is well aware of the potential drawbacks in analyzing antibody responses, and stresses the need for assessing antibody response to conformationally-dependent epitopes. The strategies which he suggests should provide information on the ability of various antigen preparations to induce conformationally-dependent antibodies with neutralizing activity. The applicant is cognizant of the need to assess the neutralizing activity of antibodies, and proposes several strategies to examine this issue. The experiments to define the immune response of animals to capsid protein preparations and to assess the ability of these preparations to elicit protection against natural and experimental infection are also straightforward.

Of particular interest is the question of maternally-derived antibodies to COPV in mother's milk. The applicant notes that a major drawback with any analysis of antibody responses to a mucosal pathogen is the lack of good data on secretory IgA responses in the dog. Hence, his suggestion to evaluate IgA secreted in milk is an interesting and useful approach for assessing secretory responses after vaccination. The studies proposed on the characteristics of the antibody response to virus preparations are, once again, straightforward and utilize technology that is readily available or adaptable.

Both models for assessing anti COPV neutralizing activity, i.e., the nude mouse and the transformation assays, possess technical difficulties. However, an assay for viral neutralization is critical for the evaluation of prospective papillomavirus vaccines, and the two proposed strategies represent reasonable approaches.

A concern with this proposal is whether there will be sufficient material to carry out the extensive studies described in the application. In particular, the question remains as to whether virions can be obtained from wart tissues in the dog colonies to set up the initial enzyme-linked immunosorbent assay (ELISA). Similarly, it is not known if the proposed expression vectors, particularly the COS cell-based expression system for L1 and L2, will provide the applicant with sufficient material for the immunization and analysis schemes outlined in the proposal. Another uncertainty is whether the recombinant material will stimulate a strong neutralizing antibody response. To a large extent, this proposal is based upon the belief that the behavior of the L1 gene product of COPV will be analogous to that of the L1 gene product of HPV type 1 (HPV 1). Despite the concerns noted, these experiments are important and should be pursued.

Aim 2, which is to sequence the L1 and L2 genes of COPV, should be given a low priority since L1 has already been sequenced and the L2 sequence is near

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Richard Schlegel, M.D., Ph.D.

completion. There may be technical difficulties in precipitating L2 and the investigator should consider alternative strategies such as co-transfection with a neo plasmid and selection with G418. It is well established that regression of warty tumors involves the cell-mediated response. As the applicant has indicated, in the case of bovine papilloma virus (BPV) infection, a combination of humoral and cellular responses may be effective. It might, therefore, be advantageous to measure appropriate responses in animals, which have been successfully immunized, or in animals in which vaccination occurs following infection, a situation that appears to enhance tumor growth.

Specific Aim 3 involves the development of monoclonal antibodies which may be directed against conformational epitopes. It will be important to identify and characterize the best epitopes for the development of peptide-derived vaccines. Furthermore, it would be of value to establish the cross reactivity of HPV antibody to 405D5 in the assay for COPV L1 expression in baculovirus.

In Aim 4, cells or nuclei containing a vector should be used as a negative control in the vaccinations, and a heterologous protein should be employed as a negative control for purified L1 or L2. If the timing of the vaccination is crucial to protection/enhancement of viral infection, it would be wise to begin those definitive studies immediately, rather than waiting until the second year. Natural infections (tumors) arise in 10-20 percent of the animals. Southern blot or polymerase chain reaction (PCR) analysis on these animals may be warranted to determine the latency rate. In any event, natural infections could complicate the interpretation of the vaccination studies.

In summary, this investigation should yield extremely valuable information for the development of a papillomavirus vaccine. Consequently, support for four years is recommended.

#### INVESTIGATORS

Dr. Richard Schlegel (20 percent effort), Principal Investigator, received a Ph.D. degree in microbiology and an M.D. degree from Northwestern University School of Medicine in 1974 and 1975, respectively. Following training in pathology at Harvard University (1980), he joined NCI as a Senior Investigator, Laboratory of Pathology (1982-1984). While at NCI, he also served as Chief, Cell Regulation and Transformation Section, Laboratory of Tumor Virus Biology (1984-1990). He is currently Associate Professor, Department of Pathology, Georgetown University School of Medicine (GUSM) and Director, Experimental Pathology Program at GUSM. Dr. Schlegel is a well respected papillomavirus virologist who has made useful contributions to the study of papillomavirus replication. Dr. A. Bennett Jenson (five percent effort, Co-Investigator, received an M.D. degree from Baylor College of Medicine in 1966. He is presently Associate Professor, Department of Pathology, GUSM. Dr. Jenson has worked extensively on the humoral immune response to papillomaviruses. Dr. Joseph Newsome (20 percent effort), Co-Investigator, received a D.V.M. degree from Ohio State University in 1986. He is currently Surgical Veterinarian and Instructor, Department of Pathology, GUSM and Manager, Research Resources Facility, GUSM. Dr. Newsome will coordinate studies on the infection of dogs and will also be involved in the work on viral neutralization

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assays. Although he lists a number of publications, his research experience appears to be somewhat limited in the area of COPV.

#### RESOURCES AND ENVIRONMENT

The facilities and resources available for maintaining the animals and for the immunological and molecular studies are appropriate.

#### BUDGET

If research progress occurs more slowly than anticipated, it is recommended that additional supply costs for the beagles in years 2 and 4 (\$28,000) be restricted for that purpose. Costs for travel are considered excessive since tissue samples could be transported by overnight courier services. Therefore, funds for travel (\$1,800) are deleted for year 1 and the duration of the proposed funding period. The remainder of the budget is appropriate as requested.

Recommendation: Support for four years.

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Richard Schlegel, M.D., Ph.D.

NATIONAL CANCER INSTITUTE  
SPECIAL REVIEW COMMITTEERFA CA-91-28  
VACCINES FOR HUMAN CANCERS OF VIRAL ETIOLOGY  
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Laboratory of Infectious Diseases  
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and Infectious Diseases  
Bethesda, MD

Robert W. Finberg, M.D.  
Associate Professor  
Department of Medicine  
Dana-Farber Cancer Institute  
Harvard Medical School  
Boston, MA

Ronald S. Ostrow, Ph.D.  
Senior Research Associate  
Institute of Human Genetics  
University of Minnesota  
Minneapolis, MN



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Jing-hsiung Ou, Ph.D.  
Assistant Professor  
Department of Microbiology  
University of Southern California  
School of Medicine  
Los Angeles, CA

Nancy Raab-Traub, Ph.D.  
Associate Professor  
Department of Microbiology  
and Immunology  
University of North Carolina  
Medical School  
Lineberger Comprehensive Cancer  
Center  
Chapel Hill, N.C.

William S. Robinson, M.D.  
Professor  
Department of Medicine  
Stanford University School  
of Medicine  
Stanford, CA

John W. Sixbey, M.D.  
Member  
Departments of Infectious Diseases  
and Virology and Molecular Biology  
St. Jude Children's Research  
Hospital  
Memphis, TN

David Smotkin, M.D., Ph.D.  
Assistant Professor  
Department of Obstetrics  
and Gynecology  
University of Utah  
School of Medicine  
Salt Lake City, UT

Bettie M. Steinberg, Ph.D.  
Head  
Section of Otolaryngology Research  
Long Island Jewish Medical Center  
New Hyde Park, N.Y.

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Richard Schlegel, M.D., Ph.D.

Michael A. Terry, B.S.  
Assistant Director for  
Administration  
Environmental Health Sciences Center  
University of Rochester  
School of Medicine  
Rochester, N.Y.

Jack R. Wands, M.D.  
Director  
Molecular Hepatology Laboratory  
Massachusetts General Hospital  
Cancer Center  
Charlestown, MA

#### SCIENTIFIC REVIEW ADMINISTRATOR

Florence E. Farber, Ph.D.  
Health Scientist Administrator  
Grants Review Branch  
Research Programs Review Section  
National Cancer Institute  
Bethesda, MD

#### PROGRAM REPRESENTATIVES

Jack Gruber, Ph.D.  
Chief  
Biological Carcinogenesis  
Division of Cancer Etiology  
National Cancer Institute  
Rockville, MD

John S. Cole, III, Ph.D.  
Program Director  
RNA Virus Studies II  
Division of Cancer Etiology  
National Cancer Institute  
Rockville, MD

Gretchen Hascall, Ph.D.  
Program Director  
DNA Virus Studies I  
Division of Cancer Etiology  
National Cancer Institute  
Rockville, MD

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Richard Schlegel, M.D., Ph.D.

May Wong, Ph.D.  
Program Director  
DNA Virus Studies II  
Division of Cancer Etiology  
National Cancer Institute  
Rockville, MD

XXConsultants are required to absent themselves from the room during the review of any application in which their presence would constitute or appear to constitute a conflict of interest.

1/ Please refer to the accompanying material for more detailed information on Initial Review Group Recommendations.

## **Curriculum Vitae for Dr. Richard Schlegel**

Associate Professor  
Department of Pathology

### **Personal Information:**

Name: C. Richard Schlegel M.D., Ph.D.

Home Address: 3 Elmwood Court  
Rockville, MD 20850  
301-340-8184

Office Address: GR10C PreClinical Science Bldg.  
Department of Pathology  
Georgetown University Medical School  
202-687-2820

Date and place of birth: August 1, 1946  
New Brunswick, New Jersey

Social Security Number: 156-40-1258

Citizenship: USA

### **Licensure:**

Medical Licenses: Massachusetts (registration number 40361; initial date of licensure: 8/1/77; expiration date: 8/1/91).

District of Columbia (pending)

### **Certification:**

Diplomat: National Board of Medical Examiners  
American Board of Pathology

Board Certification: Anatomic Pathology, 1979

## **Education:**

- 1964-1968 **B.A.**, Rutgers University, New Brunswick, New Jersey. Major in biological sciences.
- 1968-1975 **M.D./Ph.D.**, Northwestern University Medical School, Chicago, Illinois. Ph.D. in Microbiology under the supervision of Dr. Hutton Slade.
- 1975-1979 **Resident in Pathology**, Harvard Medical School, Peter Bent Brigham Hospital, Boston, Massachusetts. Chairman: Dr. Ramzi Cotran.
- 1975-1980 **Research Fellow**, Department of Pathology, Harvard Medical School, Boston, Massachusetts. Postdoctoral fellowship under the supervision of Dr. Thomas Benjamin.
- 1980-1982 **Staff Associate**, Laboratory of Molecular Biology, National Cancer Institute, NIH. Supervised by Dr. Ira Pastan.

## **Professional Experience:**

- 1982-1984 **Senior Investigator**, Laboratory of Pathology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland. Laboratory Chief: Dr. Lance Liotta.
- 1984-1990 **Chief**, Cell Regulation and Transformation Section, Laboratory of Tumor Virus Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland. Laboratory Chief: Dr. Peter Howley.
- 1988-1990 **Clinical Associate Professor**, Department of Pathology, Georgetown University School of Medicine, Washington, D.C. Acting Chairman: Dr. Bennett Jenson.
- 1990- **Associate Professor**, Department of Pathology, Georgetown University School of Medicine, Washington, D.C. Chairman: Dr. Jeffrey Cossman.
- 1991- **Director**, Experimental Pathology Program, Department of Pathology, Georgetown University School of Medicine, Washington, D.C.
- 1991- **Director**, Ob/Gyn Oncology Program (Core Grant), Lombardi Cancer Center, Georgetown University School of Medicine, Core Director: Dr. Marc Lippman.

Graduate Student Thesis Committees for:

Vivien Bubb (Pathology)  
Lex Cowser (Microbiology)  
Robert Olson (Pathology)  
Xian Wen Jin (Pathology)  
Laufey Amundadottir (Anatomy and Cell Biology)  
Thorkell Andresson (Pathology)

Annual Leadership Retreat

The Sciences and Biomedical Research at Georgetown  
September 20-21, 1991  
Chantilly, VA

Organizer

Research Seminar Series, Dept. Pathology

**Teaching Activities:**

Courses:

175-521 Neoplasia: A Survey. Lecturer, Fall Semester (1990).

175-501 Medical Pathology. Lecturer, Spring Semester (1990, 1991).

175-902 Tutorial Pathology. Lecturer, Spring and Fall Semester (1991).

175-513 Human tumor viruses and cancer. **Course director** and lecturer, Spring Semester (1992).

175-999 Thesis Research. Director for Dr. Vivien Bubb (1986-1990) and Thorkell Andresson, 1990-.

Advisor, M.D./Ph.D. Program for:

Dr. David Badawi

## **Honors and Awards:**

Phi Beta Kappa, Rutgers University, 1967;  
Summa cum laude, Rutgers University, 1968;  
Henry Rutgers Fellow, 1967-1968.  
Sigma Xi, First place in graduate student research competition, 1972.  
National Research Service Award, 5-T32-HL-07066-02, 1977  
Public Health Service Commendation Award, 1987.  
Georgetown University Medical Center Candidate for Bristol-Meyers  
Award for Distinguished Achievement in Cancer Research, 1992.

## **Professional Societies:**

American Association for the Advancement of Science  
American Society of Cell Biology  
American Society of Microbiology  
American Association of Pathologists

## **Public Service:**

### **Editorial Boards:**

Virology, Associate Editor (-1993).

### **Reviewer:**

Science, Cell, Proc. Natl. Acad. Sci. USA, EMBO J., J. Virol., Am. J.  
Path., Oncogene, Cancer Research, J. Cell. Physiol., Anal.  
Biochem., Mol. Carcinogenesis

### **NIH Review Committees:**

Site visit special review committee, 2-PO1-CA30246-07, Regulatory functions of protein/ nucleic acid interactions, March 5, 1987.

Special review committee, RFA 90-CA-09, New approaches to understanding transformation by SV40 Virus. Polyomaviruses. and Adenoviruses, November 4-5, 1990.

**NIH Advisory Committees:**

Intramural Review Panel (Tenure and Promotion)  
Division of Cancer Prevention and Control, NCI  
(Ad Hoc Member)  
Chairman: Dr. Edward Sondik, Deputy Director, DCPC  
Oct. 23, 1991.

NCI Workshop on: The Transformation Mechanisms of Papillomaviruses,  
Feb. 18-19, 1986. (RFA development).

NCI Workshop on: Prospects for Human Papillomavirus Vaccines and  
Immunotherapies, Sep. 22, 1987. (RFA development).

**Scientific Advisory Boards:**

Pharmagenics, Inc., (1990- )

**Scientific Advisor:**

Wyngate Elementary School science demonstrations and science fair,  
Bethesda, MD (1987- ).

**University Service:**

**Georgetown University Committees:**

Antisense Consortium, Georgetown University Medical School, 1990-.

Pathology Graduate Program Committee, Georgetown University  
Medical School, 1990-.

Molecular Diagnostics Committee, Georgetown University Medical  
School, 1990-.

Medical Center Task Force (Improving Clinical Research at Georgetown)  
Chairman: Dr. Paul Katz, Dept. Medicine  
Nov. 5, 1991-

Advisor, Ph.D. Graduate Program

Thorkell Andresson

## **Scholarship and Research:**

### **Funded Research Grants:**

Principal Investigator, NIH research grant 1-R01CA53371-01  
Papillomavirus E5 oncoprotein and cell transformation  
funding period: Jan 1991-Dec 1995,  
total funding: \$1,677,089.00, (60% effort).

Co-investigator, NIH research grant 1-R01-CA47624-01  
Antigenic determinants of the papillomavirus L1 capsid protein,  
funding period: July 1989-June 1992,  
total funding: \$256,000.00 (10% effort).

Sponsor, Physician scientist award 1K11CA01626-01  
Melissa Conrad  
Human papillomavirus E5 proteins  
funding period: Jan. 1992-Dec. 1996.  
total funding: \$500,106.00 (10% effort for sponsor).

### **Pending Research Grants:**

Principal Investigator, NIH grant (RFA; CA-91-28)  
Canine Oral Papillomavirus: Model for a Vaccine  
funding period: July 1992-June 1996  
total funding: \$845,625.00 (20% effort)

### **Academic/Industrial Collaborations:**

Pharmagenics Inc.  
Antisense inhibition of papillomavirus transformation  
funding: yearly renewal  
total funding: \$50,000.00/year



Publications: Original papers

1. **Schlegel, R. and Slade, H. D.:** Determination of the rate of transformation from growth curves of transformed streptococci. *J. Bacteriol.* 111: 199-202, 1972.
2. **Schlegel, R. and Slade, H. D.:** Bacteriocin production by transformable group H streptococci. *J. Bacteriol.* 112: 824-829, 1972.
3. **Schlegel, R. and Slade, H. D.:** Properties of a streptococcus sanguis (Group H) bacteriocin and its separation from the competence factor of transformation. *J. Bacteriol.* 115: 655-661, 1973.
4. **Schlegel, R. and Slade, H. D.:** Alteration of macromolecular synthesis and membrane permeability by a *Streptococcus sanguis* bacteriocin. *J. Gen. Microbiol.* 81: 275-277, 1974.
5. Duncan, J. L. and **Schlegel, R.:** Effect of Streptolysin O on erythrocyte membranes, liposomes, and lipid dispersions: a protein-cholesterol interaction. *J. Cell Biol.* 67:160-173, 1975.
6. **Schlegel, R., Schaffhausen, B., Fluck, M., Silver, J., and Benjamin, T.:** The hr-t function of polyoma virus. In *Proceedings of the EMBO-INSERM Workshop, Early Proteins of Oncogenic DNA Viruses*, Grignon, France, 69: 39-48, 1977.
7. **Schlegel, R. and Benjamin, T.:** Cellular alterations dependent upon the polyoma virus hr-t function: separation of mitogenic from transforming capacities. *Cell* 14: 587-599, 1978.
8. **Schlegel, R., Banks-Schlegel, S., and Pinkus, G.:** Immunohistochemical localization of keratin in normal human tissues. *Lab. Invest.* 42: 91-96, 1980.
9. **Schlegel, R., Banks-Schlegel, S., McLead, J., and Pinkus, G.:** Immunoperoxidase localization of keratin in human neoplasms. *Am. J. Pathol.* 101:41-48, 1980.
10. Willingham, M., Rutherford, A., Gallo, R., Wehland, J., Dickson, R., **Schlegel, R., and Pastan, I.:** Receptor-mediated endocytosis in cultured fibroblasts: cryptic coated pits and the formation of receptosomes. *J. Histochem. Cytochem.* 29: 1003-1013, 1981.
11. Banks-Schlegel, S., **Schlegel, R., and Pinkus, G.:** Keratin protein domains within the human epidermis. *Exp. Cell Res.* 136: 465-469, 1981.

12. **Schlegel, R., Willingham, M., and Pastan, I.:** Monensin blocks endocytosis of vesicular stomatitis virus. *Biochem. Biophys. Res. Commun.* 102: 992-998, 1981.
13. **Schlegel, R., Dickson, R., Willingham, M., and Pastan, I.:** Amantadine and dansylcadaverine inhibit vesicular stomatitis virus uptake and receptor-mediated endocytosis of alpha<sub>2</sub>-macroglobulin. *Proc. Nat. Acad. Sci. USA* 79:2291-2295, 1982.
14. **Dickson, R., Schlegel, R., Willingham, M., and Pastan, I.:** Reversible and irreversible inhibitors of clustering of alpha<sub>2</sub>-macroglobulin in clathrin-coated pits on the surface of fibroblasts. *Exp. Cell Res.* 140: 215-225, 1982.
15. **Dickson, R., Schlegel, R., Willingham, M., and Pastan, I.:** Binding and internalization of alpha<sub>2</sub>-macroglobulin by cultured fibroblasts. Effects of monovalent ionophores. *Exp. Cell Res.* 142: 127-140, 1982.
16. **Schlegel, R., Willingham, M. C., and Pastan, I.:** Saturable binding sites for vesicular stomatitis virus on the surface of Vero cells. *J. Virol.* 43:871-875, 1982.
17. **Dickson, R., Schlegel, R., Willingham, M., and Pastan, I.:** Involvement of Na<sup>+</sup> and HCO<sub>3</sub><sup>-</sup> in receptor-mediated endocytosis of alpha<sub>2</sub>-macroglobulin, epidermal growth factor, and vesicular stomatitis virus. *J. Cell. Physiol.* 113: 353-358, 1982.
18. **Schlegel, R., Tralka, T. S., Willingham, M. C., and Pastan, I.:** Inhibition of VSV binding and infectivity by phosphatidylserine: Is phosphatidylserine a VSV-binding site? *Cell* 32: 639-646, 1983.
19. **Schlegel, R. and Wade, M.:** Neutralized vesicular stomatitis virus binds to host cells by a different "receptor." *Biochem. Biophys. Res. Commun.* 114:774-778, 1983.
20. **Schlegel, R., Banks-Schlegel, S., Vimadala, S., and Said, J.:** Keratins in carcinoid tumors. *Lab. Invest.* 49: 511-512, 1983.
21. **Eidelman, O., Schlegel, R., Tralka, T., and Blumenthal, R.:** pH-dependent fusion induced by vesicular stomatitis virus glycoprotein reconstituted into phospholipid vesicles. *J. Biol. Chem.* 259: 4622-4628, 1984.
22. **Schlegel, R. and Wade, M.:** A synthetic peptide corresponding to the NH<sub>2</sub>-terminus of vesicular stomatitis virus glycoprotein is a pH-dependent hemolysin. *J. Biol. Chem.* 259: 4691-4694, 1984.

23. **Schlegel, R. and Wade, M.:** Biologically active peptides of the vesicular stomatitis virus glycoprotein. *J. Virol.* 53: 319-323, 1985.
24. Yee, C., Krishnan-Hewlett, I., Baker, C. C., **Schlegel, R.**, and Howley, P.M.: Presence and expression of human papillomavirus sequences in human cervical carcinoma cell lines. *Am. J. Path.* 119: 361-366, 1985.
25. **Schlegel, R.:** Papillomaviruses and cervical cancer. *Innov. In Oncol.* 13-14, November, 1985.
26. **Schlegel, R.**, Glass, M., Rabson, M. S., and Yang, Y.-C.: The E5 transforming gene of bovine papillomavirus encodes a small, hydrophobic polypeptide. *Science* 233: 464-467, 1986.
27. Burkhardt, A., DiMaio, D., and **Schlegel, R.:** Genetic and biochemical definition of the bovine papillomavirus E5 transforming protein. *EMBO J.* 6: 2381-2385, 1987.
28. Zhang, Y.-L., Lewis, A., Wade-Glass, M. J., and **Schlegel, R.:** Levels of bovine papillomavirus RNA and protein expression correlate with variations in the tumorigenic phenotype of hamster cells. *J. Virol.* 61: 2924-2928, 1987.
29. **Schlegel, R.:** Papillomaviruses. *Arch. Dermatol.* 123: 537, 1987.
30. McBride, A. A., **Schlegel, R.** and Howley, P. M.: The carboxy-terminal domain shared by the bovine papillomavirus E2 transactivation and repressor proteins contains a specific DNA binding activity. *EMBO J.* 7: 533-539, 1988.
31. Bubb, V., McCance, D.J., and **Schlegel, R.:** DNA sequence of the HPV-16 E5 ORF and the structural conservation of its encoded protein. *Virology* 163: 243-246, 1988.
32. Howley, P. M., and **Schlegel, R.:** The human papillomaviruses: An Overview. *Am. J. of Med.* 85: 155-158, 1988.
33. **Schlegel, R.**, Phelps, W., Zhang, Y. L., and Barbosa, M.: Quantitative keratinocyte assay detects two biological activities of human papillomavirus DNA and identifies viral types associated with cervical carcinoma. *EMBO J.* 7: 3181-3187, 1988.
34. Horwitz, B. H., Burkhardt, A., **Schlegel, R.**, and DiMaio, D.: 44 amino acid E5 transforming protein of bovine papillomavirus requires a hydrophobic core and specific carboxyl-terminal amino acids. *Mol. Cell. Biol.* 8: 4071-4078, 1988.

35. Burkhardt, A., Willingham, M., Gay, C., and **Schlegel, R.**: The papillomavirus E5 oncoprotein is oriented asymmetrically in Golgi and plasma membranes. *Virology* 170: 334-339, 1989.
36. Munger, K., Phelps, W.C., Bubb, V., Howley, P.M., and **Schlegel, R.** The E6 and E7 genes of the human papillomavirus type 16 together are necessary and sufficient for transformation of primary human keratinocytes. *J. Virol.* 63: 4417-4421, 1989.
37. Barbosa, M., and **Schlegel, R.**: The E6 and E7 genes of HPV-18 are sufficient for inducing two-stage in-vitro transformation of human keratinocytes. *Oncogene* 4: 1529-1532, 1989.
38. Goldstein, D., and **Schlegel, R.**: The E5 oncoprotein of bovine papillomavirus binds to a 16 kilodalton cellular protein. *EMBO J.* 9: 137-146, 1990.
39. Pietenpol, J.A., Stein, R.W., Moran, E., Yaciuk, P., **Schlegel, R.**, Lyons, R.M., Pittelkow, M.R., Munger, K., Howley, P.M., and Moses, H.L.: TGF-beta1 inhibition of c-myc transcription in keratinocytes is abrogated by DNA tumor virus transforming proteins with intact binding domains for the retinoblastoma gene product. *Cell* 61: 777-785, 1990.
40. Villa, L., and **Schlegel, R.**: Differences in transformation activity between HPV-18 and HPV-16 map to the viral LCR-E6-E7 region. *Virology* 181: 374-377, 1991.
41. Romanczuk, H., Villa, L.V., **Schlegel, R.**, and Howley, P.: The differential immortalization activities of human papillomavirus types 16 and 18 are determined by viral transcriptional regulatory regions upstream of the E6 and E7 genes. *J. Virol.* 65: 2739-2744, 1991.
42. Goldstein, D., Finbow, M.E., Andresson, T., McLean, P., Smith, K., Bubb, V., and **Schlegel, R.**: The bovine papillomavirus E5 oncoprotein binds to the 16 kilodalton component of vacuolar H<sup>+</sup>-ATPases. *Nature* 352: 347-349, 1991.
43. Bernstein, E.F., Glass, J.M., DeGraff, W.G., **Schlegel, R.**, Black, C., Fisher, J.M., Cook, S.N., Glatstein, E., Russo, A., and Mitchell, J.B.: In-vitro photodynamic treatment of normal and human papillomavirus-transfected keratinocytes with photofrin II and red light. *Arch. Dermatol.* 127: 683-687, 1991.
44. Goldstein, D.J., Kulke, R., DiMaio, D., and **Schlegel, R.**: A glutamine residue in the membrane-associating domain of the bovine papillomavirus type 1 E5 oncoprotein mediates its binding to a transmembrane component of the vacuolar H<sup>+</sup>-ATPase. *J. Virol.* 66: 405-413, 1992.

45. Toyama, R., Goldstein, D.J., **Schlegel**, R., and Dhar, R.: A genomic sequence of the *Schizosaccharomyces pombe* 16 kD vacuolar H<sup>+</sup>-ATPase. *Yeast* 7: 989-991, 1992.
46. Villa, L.L., Vieira, K., Pei, X.-F., and **Schlegel**, R.: Differential effect of tumor necrosis factor on the proliferation of primary human keratinocytes and cell lines containing human papillomavirus types 16 and 18. *Mol. Carcinogenesis* (in press).
46. Yankaskas, J., Haizlip, J., Conrad, M., Koval, D., Lazarowski, E., **Schlegel**, R., and Boucher, R.: Cystic fibrosis tracheal epithelial cells immortalized by HPV-18 oncogenes retain a differentiated phenotype and biochemical defect. *J. Clin. Invest.* (submitted).
47. Ghim, S., Jenson, A. B., and **Schlegel**, R.: HPV-1 L1 protein expressed in cos cells displays conformational epitopes found on intact virions. *J. Virol.* (submitted).
48. Goldstein, D., Toyama, R., Dhar, R., and **Schlegel**, R.: The BPV-1 E5 oncoprotein expressed in *Schizosaccharomyces pombe* exhibits normal biochemical properties and binds to a conserved 16 kDa component of the vacuolar proton-ATPase. (*Virology*, submitted).

Publications: Reviews and chapters.

1. **Schlegel, R.:** Membrane-active peptides of the vesicular stomatitis virus glycoprotein. In Lonberg-Holm, K. and Crowell, R. L. (Eds.): Virus Attachment and Entry into Cells. Washington, D. C., ASM Publications, 66-73, 1986.
2. Howley, P. M. and **Schlegel, R.:** Papillomavirus transformation. In N. P. Salzman and P.M. Howley (Eds.): Papovaviridae. 2. The Papillomaviruses, New York, Plenum Press, 1986, pp. 141-166.
3. **Schlegel, R.:** Probing the function of viral fusion proteins with synthetic peptides. In A. Sowers (Ed.) Cell Fusion, New York, Plenum Press, 33-43, 1987.
4. **Schlegel, R., and Wade-Glass, M.:** The E5 transforming protein of bovine papillomavirus. In Steinberg, B., Brandsma, J., and Taichman, L. (Eds.): Cancer Cells, New York, Cold Spring Harbor Laboratory Press, 1987, pp. 87-91.
5. Phelps, W.C., Munger, K., Yee, C.L., **Schlegel, R., and Howley, P.M.:** The genital human papillomaviruses: transcriptional regulation and transformation. In: Common mechanisms of transformation by small DNA tumor viruses. Ed. L.P. Villareal, ASM Publications, Washington, DC, pages 149-164, 1989.
6. Munger, K., Phelps, W.C., Bubbs, V., Howley, P.M., and **Schlegel, R.:** Keratinocyte transformation by the HPV-16 E6 and E7 genes progresses through two distinct morphological stages. In: Papillomaviruses, UCLA Symposium on Molecular and Cellular Biology, New Series, Volume 124, pages 223-230, Editors, P. Howley and T. Broker, Alan R. Liss Inc., New York, NY, 1989.
7. **Schlegel, R.:** Papillomaviruses and human cancer. In: Viral pathogenesis (ed. Fujinami, R.), Seminars in Virology 1: 297-306, 1990.
8. Howley, P.M., Munger, K., Werness, B., Phelps, W.C., and **Schlegel, R.:** Molecular mechanisms of transformation by the human papillomaviruses, Genetic basis for carcinogenesis: tumor suppressor genes and oncogenes, A.G. Knudson, Jr. et.al. (eds.), Japan Sci. Soc. Press, Tokyo/Taylor and Francis Ltd., London pp 199-206, 1990.
9. Cossman, J., and **Schlegel, R.:** p53 in the diagnosis of human neoplasia. JNCI 83: 87-88, 1991.

10. Finbow, M., Pitts, J., Goldstein, D., **Schlegel, R.**, and Findlay, J.: The E5 oncoprotein target: a 16 kD channel-forming protein with diverse functions. *Molecular carcinogenesis* (in press).
11. Conrad, M., Yankaskas, J., Boucher, R., and **Schlegel, R.**: Using the papillomavirus E6/E7 genes to generate well-differentiated epithelial cell lines. in: *Neoplastic transformation in human cell systems in vitro: mechanisms of carcinogenesis*, Humana Press (in press).

## Meetings:

### Invitations to International Meetings (since 1988):

1. Third Conference on Differentiation Therapy. Speaker. Modulation of keratinocyte proliferation and differentiation: two dissociable activities of the human papillomaviruses. September 5-10, 1988, Villasimius, Sardinia.
2. 1989 UCLA Symposium on "Papillomaviruses". Symposium Speaker. Modulation of keratinocyte differentiation by human papillomaviruses. March 11-18, 1989. Taos, New Mexico.
3. Papillomavirus Workshop 1990. Oral presentation. The E5 oncoprotein of bovine papillomavirus bind to a 16 kd cellular protein. May 12-17, 1990, Heidelberg, Germany .
4. International Symposium on Human Papillomavirus. Plenary session speaker. Different HPV genotypes have different transforming activities. November 8-10, 1990, Sao Paulo, Brazil.
5. International Papillomavirus Workshop. Plenary session speaker. HPV Transformation: Host-Cell Interactions. July 20-26, 1991, Seattle, Washington.
6. 11<sup>th</sup> International Papillomavirus Workshop, Session Chairman and Plenary session speaker, Edinburgh, Scotland, Sept. 5-12, 1992.



Invitations to Domestic Meetings and Lectures (since 1989):

1. Georgetown University, Department of Obstetrics and Gynecology, A quantitative in-vitro assay for the biological activity of human papillomaviruses. January 27, 1989.
2. University of Tennessee Medical School, Department of Pathology, Memphis, TN, Cellular transformation by human papillomaviruses. February 27-28, 1989.
3. University of Connecticut Health Center, Department of Microbiology, Keratinocyte transformation by papillomaviruses and the role of the E5 oncoprotein. February 22, 1989.
4. E.I. DuPont., Delaware, Keratinocyte transformation by the human papillomaviruses. April 19, 1989.
5. Armed Forces Institute of Pathology, Division of Molecular Diagnostics, Washington, DC, The role of human papillomaviruses in human neoplasia. May 16, 1989.
6. Triton Biosciences, Inc., Oakland, CA, Papillomavirus oncoproteins. June 5, 1989.
7. Harvard School of Public Health, Department of Toxicology, Boston, MA, Viruses and human neoplasia. February 5, 1990.
8. Georgetown University, Department of Microbiology and Immunology, HPV genes involved in human keratinocyte transformation. September 26, 1990.
9. Lombardi Cancer Center, Georgetown University Medical School, HPV and human neoplasia. November 30, 1990.
10. Georgetown University Medical School, Department of Obstetrics and Gynecology, Biological differences between HPV-16 and HPV-18, November 26, 1990.
11. Roswell Park Cancer Institute, Buffalo, NY, The transforming oncoproteins of the papillomaviruses. March 3-5, 1991.
12. Georgetown University Medical Center, A Workshop on Neoplastic Transformation in Human Cell Systems In-Vitro: Mechanisms of Carcinogenesis, The phenotype of human epithelial cells immortalized by the E6/E7 oncogenes of HPV-18, April 25-26, 1991.

13. Gordon Conference on Epithelial Differentiation and Keratinization, Tilden School, New Hampshire, Altered tumor suppressor gene expression in HPV-transformed keratinocytes, July 28-August 2, 1991.
15. American Medical Association and Georgetown University Medical School, Georgetown University Medical School, Polymerase Chain Reaction--A Diagnostic Tool for the 1990's, moderator, October 11-12, 1991.
16. Gordon Research Conference, Drug Carriers in Biology and Medicine, Molecular approaches to the prevention and treatment of HPV infections, New Hampshire, July 6-10, 1992.
17. Fifth Annual HPV Conference, Chicago, IL, Speaker, The biology of the human keratinocyte, Oct. 25-28, 1992.